(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 108 472 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
20.06.2001 Bulletin 2001/25

(51) Int Cl.[7]: **B01L 7/00**, B01L 3/00, B01J 19/00

(21) Application number: **00302224.1**

(22) Date of filing: **17.03.2000**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **15.12.1999 JP 35643399**

(71) Applicant: **Hitachi, Ltd.**
Chiyoda-ku, Tokyo 101-8010 (JP)

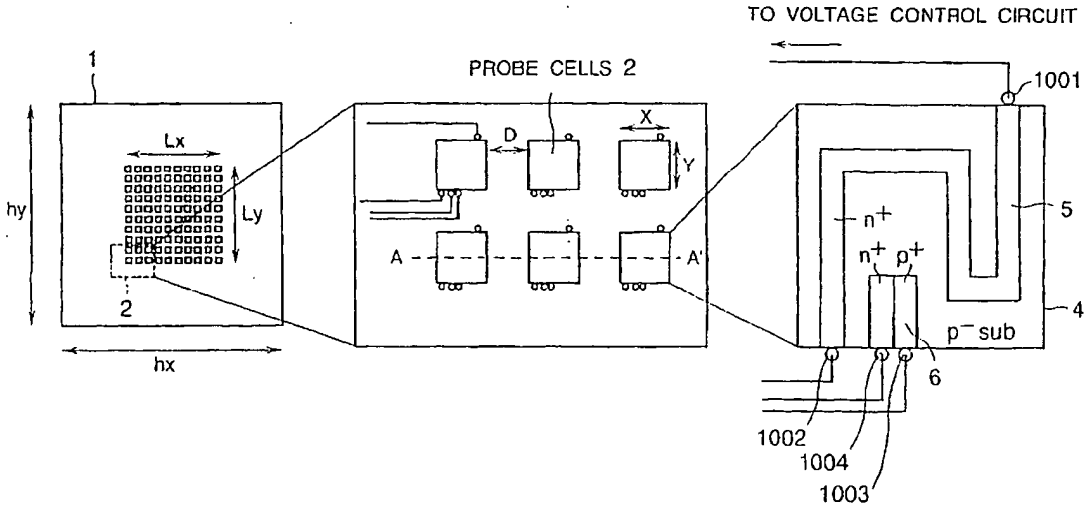
(72) Inventors:
- **Kajiyama, Tomoharu**
  Kokubunji-shi, Tokyo 185-0005 (JP)
- **Miyahara, Yuji**
  Kodaira-shi, Tokyo 187-0022 (JP)
- **Murakawa, Katsuji**
  Kodaira-shi, Tokyo 187-0045 (JP)

(74) Representative: **Nicholls, Michael John**
J.A. KEMP & CO.
14, South Square
Gray's Inn
London WC1R 5JJ (GB)

(54) Advanced thermal gradient DNA chip (ATGC), it's manufacture method and method for carrying out biochemical reactions

(57) A biochemical reaction detection chip capable of controlling the temperature for biochemical reactions including hybridizations and its substrate. The function of the chip is performed by comprising a plurality of islands of a heat conducting material on the membrane of the substrate, the islands being spaced from each other and individually provided with temperature controllers, and the probes immobilized on the substrate.

FIG.1A FIG.1B FIG.1C



EP 1 108 472 A2

[0010] Further another object of the present invention is to provide a substrate of a biochemical reaction detection chip comprising a plurality of islands of a heat conductive material formed on a membrane, the islands being placed apart from each other and each island being provided with a temperature controller.

[0011] It is preferable for the membrane to be formed from a material having a high insulating ability, heat insulating ability and physical strength. The electric conductivity of 10^8 Ω · m or more is sufficient for the membrane material, preferably, 10^{10} Ω · m or more. The heat conductivity of 10w/mk or less is sufficient for the membrane material, preferably, 1w/mk or less.

[0012] It is easier to control the temperature of each island by forming the membrane from a material having a high (electrical) insulating ability and a high heat insulating ability. The membrane may be formed, for example, from at least one of a group of materials such as silicon nitride, silicon oxide, aluminum oxide, Ta_2O_5, or may be a composite membrane of these materials. Among these, the composite membrane of SiN and SiO_2 is preferable. Since SiN has resistance to alkali, probes can be immobilized on SiN membrane by means of silane coupling in alkali solution. Further, the SiN membrane is capable of protecting the electronic circuit for temperature control provided thereunder from the solution such as sample solution.

[0013] The film thickness of 1-500 μm is sufficient, preferably, 5-20 μm.

[0014] It is preferable to make an indent for the area for fixing the probe of the membrane. Such indent is convenient for holding the sample solution on a chip when letting the biochemical reaction take place by bringing sample solution into contact with the probe.

[0015] Further, a resist membrane may be formed on the surface opposite to the islands. The resist membrane may be of a photosensitive polyimide resin or the like.

[0016] A plurality of islands of a heat conductor are formed on the membrane. "A plurality of islands" means at least 2 islands, preferably 10-1000 islands, although the number of the islands is not defined. A plurality of islands may be arranged either in line or 2-dimensionally, that is, in a first direction (row) and a second direction (column).

[0017] The islands are formed from a heat conductor. Examples of heat conductors include crystals of Si, metals such as Ag, Au, Cu and silicones such as polysilicone and amorphous silicone. The heat conductor constituting the islands is preferable to be electrically insulatable from the temperature controller. Silicone is preferable as a heat conductor to form the islands, since it is a good heat conductor and can be electrically insulated from the temperature controller. The insulation between the heat conductor and the temperature controller can be secured by forming a pn junction in the silicone.

[0018] The islands are spaced from each other. The spaces among the islands serve as a substitute for heat insulating material, and so the temperature of each island can easily be controlled independently.

[0019] The size of 10-1000 μm^2 is sufficient for an island, preferably 50-500 μm^2 . The interval of 50.1000 μm between islands is sufficient, preferably 100-500 μm. The shape of islands are not defined specifically. For instance, when forming the islands of Si crystal from a flat sheet of Si crystal having 100 planes as a surface by removing unnecessary portion by etching with KOH, 111 planes are exposed during manufacturing process, making a regular pyramid-like form.

[0020] Each of a plurality of islands is provided with the temperature controller. More particularly, it is preferable to provide a heating circuit and a temperature detection circuit for each island. The heating circuits and the temperature detection circuits may be controlled to operate independently either for each island or for each group of islands.

[0021] Further, where a plurality of islands are arranged twodimensionally, the heating circuits and the temperature detection circuits may be controlled to operate independently for each (first or second) line. The size of the biochemical reaction detection chip is sufficient to be 25 mm^2-100 cm^2, preferably 100 mm^2 -14 cm^2.

[0022] With a biochemical reaction detection chip manufactured by immobilizing probes on a substrate of the biochemical reaction detection chip according to the present invention, the influence of the temperature of an adjacent probe cell (reaction system) can be reduced so that the biochemical reaction is allowed to progress at a proper temperature on each of the probe cells (reaction system).

[0023] The substrate for the biochemical reaction detection chip according to the present invention is preferable to be provided with heat sinks for allowing heat to escape outside installed among the islands. Each heat sink is preferable to have a structure (e.g., a mesh structure) that prevents it from directly contacting with islands. The heat sinks may be installed either only for one direction or both directions of first and second directions. Where the probes are divided into groups according the proximity of optimal temperatures of biochemical reactions and fixed on the membrane, heat sinks may be provided for each area of such groups, respectively.

[0024] It is preferable to form heat sinks from materials having good heat conductivity, such as Si, Au, Ag, Cu and the like.

[0025] Forming heat sinks among the islands enables heat to escape outside before being transmitted from any adjacent islands.

[0026] The distance between an island and a heat sink is sufficient to be 10-500 μm, preferably 10-250 μm.

[0027] Further, the present invention relates to a method for manufacturing the substrate for the biochemical reaction detection chip, more particularly to a method comprising the steps of:

in each reaction system and maintaining the temperature for a certain period of time.

(2) A method according to (1) above, wherein the heating process (a) is carried out in an incubator.

(3) A method according to (1) above, wherein the process for lowering the temperature (b) is carried out by stopping heating process (a) or using a cooler.

(4) A method according to (1) above, wherein the biochemical reaction is the hybridization between polynucleotide and oligonucleotide, and the optimal temperature for the biochemical reaction is the melting temperature of double strand formed with the oligonucleotide and its complementary strand.

(5) A method according to (4) above, wherein the polynucleotide is DNA in a sample, and the oligonucleotide is oligonucleotide probe of the biochemical reaction detection chip.

Further, the present invention also provides a storage medium storing a program for performing the biochemical reaction controlled by a computer.

(6) A computer-readable strage medium storing a program for executing a method for performing a plurarity of biochemical reactions in a plurarity of reaction systems simultaneously while controlling the temperature for each reaction system, the method comprising the steps of:

(a) heating all the reaction systems to a temperature higher than the optimal temperature for the biochemical reaction in each reaction system, and
(b) lowering the temperature of each reaction system to an optimal temperature for each biochemical reaction in each reaction system and maintaining the temperature for a certain period of time.

[0042] When the optimal temperature for a biochemical reaction is the melting temperature of double strand formed with oligonucleodide probe and its complementary strand, the temperature higher than the optimal temperature for the biochemical reaction is preferably a temperature at which the double stranded nucletiode dissociates completely, for example, a temperature between 90°C-99 °C. The temperature suitable for the biochemical reaction may be a temperature around the melting temperature, e.g., within the melting temperature ± 2°C.

[0043] An embodiment of the present invention will be described in the following. A sample is injected into reaction systems on a biochemical reaction detection chip. Then, the chip is covered and placed in an incubator and heated to a maximum temperature, e.g., 90°C. Normally, the incubator is provided with a heater and a cooler so that the internal temperature can be adjusted to a predetermined temperature. The temperature of the incubator is then set to a minimum temperature, e.g., 15 °C, to bring down the temperatures of all the reaction systems. When the temperature of each reaction system (e.g., a probe cell) is become lower than a set temperature (e.g., a melting temperature of double strand formed with each probe and its complementary strand), the heater is turned on to proceed the biochemical reaction while maintaining the set temperature for each reaction system for a period of time (e.g., 12 hours). After the reaction, the reaction system (e.g., the probe cell) is washed, and the biochemical reaction is detected to process the data obtained as a result of the detection.

[0044] For detection, a fluorescent marker is generally bound to a sample so that the amount of fluorescence of the marker bound to the probe can be measured with a co-focal-point microscope, and the amount of the bonded sample is calculated on the basis of the amount of the fluorescence.

[0045] Normally, biochemical reactions started at an optimal temperature for the biochemical reactions only by heating the reaction systems. However, this method frequently results in the probe binding with a substance other the subject that to be detected, causing the noise in detecting the subject. Therefore, raising the temperature of reaction systems to a level higher than the optimal temperature for the biochemical reactions and then lowering to the optimal temperature can reduce the probability that the probe is bound with a substance other than the subject of detection, thereby reducing the noise in detecting the subject.

[0046] For the hybridization of the oligonucleotide probe and polynucleotide, the optimal temperature is the melting temperature of the double strand formed with the probe and its complementary strand. When the reaction is allowed to proceed at the melting temperature only by heating the reaction system, the oligonucleotide probe may bind with a nucleotide other than the nucleotide having the complementary strand to the probe (the subject of detection), resulting in so-called mismatching that causes the noise in detecting the subject. However, when the temperature of the reaction system is once raised to a level higher than the melting temperature, and then lowered to the melting temperature, the probability that the probe is bound with a nucleotide other than the nucleotide having the complementary strand to the probe is reduced, thereby contributing to the decrease of the noise in detecting the subject. In the method according to the present invention, the temperatures of all the reaction systems are first raised to the levels higher than the optimal

Detailed Description of the Invention

[0070] The components of the present invention and corresponding reference numerals will be described in the following. 1, the substrate for DNA chip: 2, probe cell; 4, island: 5, heater circuit; 6, pn-junction temperature detection element; 1001, heater terminal (+); 1002, heater terminal (-); 1003, temperature detection terminal (+);1004, temperature detection terminal (-); 21, Si island; 22, SiN/SiO_2 membrane; 24, temperature set up area; 25, probe; 26, sample solution; 27, cover; 41, mesh structure (heat sink); 51, sample buffer; 52, acrylic resin plate; 61, metal frame; 71, n-type substrate; 72, p-well; 73, p-well; 74, sio_2 membrane; 75, n-type diffuse layer; 76, n-type diffuse layer; 77, n-type diffuse layer; 78, p-type diffuse layer; 79, p-type diffuse layer; 81, isolation between the first layers; 82, wiring in the first layer; 83, isolation between the second layers; 84, wiring in the second layer; 91, si_3N_4 membrane; 101, DNA chip; 102, printed circuit board; 103, holder; 104, cable; 105, controller; 106, incubator; 107, fan; 108, cooling unit; 109, switch; 110, voltmeter; 111, output controller; Vpo, power source of heater; Vc, constant-voltage power source; 801, common wiring of sensor; 802, positive terminal of pn junction temperature sensor; 803, electrode; 804, electrode; 805, common electrode of sensor; 806, common electrode of heater; 901, side of island.

[0071] The embodiments of the present invention will be described referring to the drawings.

[Example 1: Structure of Substrate for DNA Chip]

[0072] Fig. 1 is a diagram schematically illustrating a substrate for biochemical reaction detection chip for immobilizing oligonucleotide DNA (hereinafter referred to as substrate for DNA chip). The substrate for DNA chip immobilized probe is called a DNA chip.

[0073] Fig. 1A is a plan view a substrate for DNA chip carrying 100 probe cells in total, comprising 10 rows (in horizontal direction) and 10 columns (in vertical direction). The substrate for DNA chip is preferable to have vertical length (hy) and horizontal length (hx) of 10-100 mm respectively. The distance Lx from the left-end of the 1st probe cell to the right-end of the 10th probe cell in horizontal direction, and the distance Ly from the upper end of the first probe cell to the lower end of the 10th probe cell in vertical direction are preferable to be 5-100 mm respectively.

[0074] Fig. 1B is an enlarged view of the framed area of Fig. 1A. The width X and length Y of each probe cell on the substrate for DNA chip for immobilizing probes are preferable to be 10-1000 μm respectively. The intervals among the probe cells are preferable to be 50-1000 μm respectively.

[0075] An island is formed under each probe cell. Fig. 1C is a partially enlarged view of the probe cell 2 of Fig. 1B. Each probe cell is provided with a heater circuit 5 formed with n-type diffuse layer, and a temperature detection element 6 formed with pn junction between p-type diffuse layer and n-type diffuse layer. A heater terminal (+) 1001 and a heater terminal (-) 1002 are formed at both ends of the heater circuit 5. When a voltage is applied across both terminals so that the 1001 side is to be a positive electrode, current flows in the n-type diffuse layer 5 to produce Joule heat. The amount of Joule heat can be controlled by controlling either the level or duration of applied voltage. The temperature detection element 6 is provided with the temperature detection terminal (+) 1003 connected to the p-type diffuse layer and the temperature detection terminal (-) 1004 connected the n-type diffused layer. The current-voltage characteristics of pn junction of the temperature detection element 6 is largely dependent on the temperature of the pn junction. Therefore, the temperature of the pn junction can be determined by detecting the current-voltage characteristics between elements. Further, since the island 4 is made of a thermal conductor, the temperature of the pn junction and the temperature of the island 4 are almost equal to each other, and thus the temperature of the probe cell 2 on the island 4 can be detected by measuring the current-voltage characteristics between pn junction elements. The temperature dependency of the current-voltage characteristics at pn junctions, for example, in the case where the voltage is fixed in a forward bias with 1003 as being positive, the flow of the current varies exponentially with the temperature of the pn junction. Alternatively, when the current is fixed in a forward bias, the temperature and potential difference can be approximated on the basis of the linear function.

[0076] Fig. 18 shows another embodiment of the heater circuit and the temperature detection element of a probe cell. In this embodiment, an n-type substrate (n-sub) is used, and the heater circuit 5 and the temperature detection element 6 on a probe cell are separated by means of separate p-wells in order to make the heater circuit and the pn junction element electrically independent. This arrangement is designed for preventing the electrical interference between the n-type diffuse layer and the temperature detection element 6. As shown in Fig. 18, the probe cell is connected to controller 105 which comprises heater power source circuit 181 and temperature detection circuit 182. The controller 105 is an example of a circuit to detect the temperature of a probe cell and control heating by the heater. The heater power source circuit 181 comprises heater power source Vp, output controller 111 and switch 109, and is connected to terminals 1001 and 1002 of the probe cell. By controlling the heater power source Vp and the output controller 111, the voltage and the current across the terminals 1001 and 1002 of the probe cell, and therefore the Joule heat occurring in the heater circuit 5 of the probe cell, may be controlled. Temperature detection circuit 182 comprises power source Vc, resistance R and voltmeter 110. Terminal 1003 is set to zero potential, and terminal 1004 is connected to negative

to the probe, the probe can be immobilized on the membrane by means of silane coupling.

[0088] Sample solution 26 is preferably added in the amount sufficient for making the solution layer with a thickness of 10-1000 μm. After addition of sample solution 26, a glass cover 27 is placed thereon.

[0089] Fig. 3 shows an example of the temperature setting for DNA chip. The temperature of each island is set to 15-90 °C. As in Fig. 3 showing the arrangement of the probe cells for temperature setting, the probes may be arranged according to their Tm value. For example, probes may be arranged so that those with higher Tm values are placed in central area. while those with lower Tm values are placed toward peripheral area. Alternatively, the probes may be arranged from one side of the chip to the other side, by placing probes from those with highest Tm to those with lowest Tm. By arranging the probes in this manner, a good balance between the dispersion and supply of the heat can be maintained for easier temperature control.

[0090] Fig. 4 is a diagram illustrating the shapes of the islands and the mesh structure formed on the membrane.

[0091] Fig. 4A is an enlarged backside picture of a DNA chip substrate. Besides the Si island 21, the mesh structure 41 is formed on the SiN/ Si_2 membrane 22 between the islands.

[0092] Fig. 4B is a cross section view at B-B' in Fig. 4A. The peak of Si constituting the mesh structure is about 250 μm high and about 350 μm wide. In this embodiment, the angle of inclination of the inclined side of the peak of Si is about 55 °. The distance between the peak of Si constituting the mesh structure and the Si island 21 is about 75 μm.

[0093] With thermal conductor layer (mesh structure) 41 formed among the islands 21, it becomes possible to make the heat of any islands escape before being transmitted to an adjacent island. That is, the mesh structure serves as a heat drain.

[0094] Fig. 5 is a diagram illustrating the effect of the mesh structure. The conditions for producing this effect is as described in the following. The width and the length of the Si island 21 are 500 μm, respectively. The height of the Si island 21 is 250 μm. Both sides of the base of the Si island 21 is 150 μm. On the other hand, the height and the width of the Si constituting the mesh structure are about 250 μm and 350 μm, respectively. The distance between the peak of Si constituting the mesh structure and the Si island 21 is about 75 μm. The thickness of SiN/SiO_2 membrane 22 is 5 μm. The thickness of the water layer 51 (e.g. sample buffer) is 20 μm, The thickness of acrylic resin plate is 5 μm.

[0095] The heat conductivity from point A (the center of the base of Si island) to point B (the center of the base of an neighboring Si island) and the heat conductivity from the point A to point C (the point 2 mm apart from the middle pointM between the point A and the point B) are compared. The heat conductivity from the point A to the point M is the same in both cases, and thus omitted from the comparison. Comparing the heat UMB transmitted from the point M to the point B per unit time with the heat UMC transmitted from the point M to the point C per unit time, where the heat transfer coefficient of the membrane is 10, and that of the Si layer is 150, the relationship of the UBC and UMC can be expressed as below.

UMB : UMC
= 10 x (Sectional area of membrane)/150
: 150 x (Cross sectional area of mesh structure)/2000
= 10 x (5 x 500)/150 : 150 x (175 x 250)/2000
= 1 : 20

[0096] Consequently, it can be found that the heat conduction from the point M to the point C is about 20 times as much as the heat conduction from the point M to the point B.

[0097] Fig. 6 is a diagram illustrating an embodiment provided with the cooling function on the periphery of the mesh. For example, a DNA chip substrate 1 is fit in metal frame 61 which is connected to a cooling unit. By providing the cooling function on the periphery of the mesh structure, the heat drain effect can be increased.

[Example 2: Chip Manufacturing Process]

[0098] Referring to Fig. 7 through Fig. 9, the manufacturing process of the DNA chip substrate, on which the islands are formed in the mesh structure on a composite membrane of SiO_2 and SiN, will be described.

[0099] Fig. 7 is a diagram illustrating the first manufacturing process of the DNA chip substrate. In this embodiment, an n-type Si substrate (N-sub) 71, having plane (100) as a surface area and thickness of 500 μm, is used as a substrate. After forming p-well pattern with the SiO_2 membrane 74 on the surface of the substrate, p-wells (1018 pieces/cm^3) 72 and 73, having a depth of 3 μm, respectively, are formed by B doping and diffusion, in order to electrically insulate the temperature detection element 6 to be formed later by n + diffusion and heater circuit 5. An SiO_2 membrane 74 is formed as a mask for separating elements and for diffusion. A dope such as boric acid is used for the p-well diffusion. Then, after forming a circuit pattern with SiO_2 membrane, a high-concentration of n-type diffuse layers 75, 76 and 77 (n +, 1020 pieces/cm^3) and a high-concentration of p-type diffuse layers 78 and 79 (p +, 1020 pieces /cm^3), with the depth of 100 nm respectively, are formed by the diffusion of phosphor (a high-concentration of n-type impurity). The n + (n-type diffuse layer) 76 constitutes the temperature detection element 6. The n + (n-type diffuse layer) 77 constitutes the heater circuit 5. The n + (n-type diffuse layer) 75 is a reference electrode terminal 75 of the n-type substrate. The

[Example 4: Temperature Control]

[0110] The apparatus for controlling the DNA chip will be described in the following.

[0111] Fig. 10 is a diagram illustrating an example of the biochemical reaction detection apparatus. DNA chip 101 comprises probe cells, each provided with a heater terminal (+) 1001, a heater terminal (-) 1002, a temperature detection terminal (+) 1003 and a temperature detection terminal (-) 1004, and wiring for these terminals and controller 105 is similar to that shown in Fig. 18. The controller 105 comprises a temperature detection circuit 182, including a voltmeter 110, a power source Vc and a resistance R, and a heater power source circuit 181, including a heater power source Vpo, an output controller 111 and a switch 109, both of which are equivalent in number to the number of probe cells to be temperature-controlled independently from one another. Fig. 18 shows only one set of such components. Wirings to these terminals are connected to a holder 103 through a printed circuit board 102, and the holder 103 is further connected to a controller 105 through a cable 104. The wiring for the terminal connected to the grounding terminal on the side of the controller 105 can be used as a common wiring for a plurality of probe cells, for simplicity.

[0112] The temperatures of the individual probe cells can be controlled independently according to the method described above reffering to Fig. 18. The temperature of each probe cell can be determined by measuring the potential difference of the incorporated temperature detection element. The level of voltage to be applied across the heater is controlled by ON/OFF operation of switch 109 according to the measured value of the temperature. When the temperature detected from the temperature detection circuit is lower than the predetermined value, the switch 109 and the output controller 111 control the output from the power source of the heater V_{po} to let the current flow in the heater. Controlling is performed to each probe cell independently.

[0113] The DNA chip 101, the printed circuit board 102 and the holder 103 are operated in a incubator 106, and a fan 107 is used when necessary for cooling the DNA chip 101 by sending the wind from below. The cooling unit 108 may be used for cooling the periphery of the DNA chip. In this embodiment, as will be described later, the temperature of the incubator is adjusted to a minimum value of various set temperatures required by the chip, and the cooling unit 108 and/or the fan 107 are used depending on the degree of the temperature raising of the chip or the temperature distribution among the probe cells arranged close to each other.

[0114] Further, the temperature of the DNA chip may be controlled with a computer. In this case, the computer serves as a temperature controller by incorporating a temperature control program which is stored in a computer-readable strage medium. The storage medium may be any type of storage medium such as RAM, ROM, magnetic disk, CD-ROM, magnetic tape, IC card.

[Example 5: Measurement]

[0115] In this example, a measurement of a DNA fragment of 17-base length with 4 kinds of probes of 8-base length will be explained.

[0116] SEQ ID NO: 1 is a DNA fragment of 17-base length (hereinafter referred to as sample DNA).

TGACCGGCAGCAAAATG (SEQ ID NO: 1)

[0117] This sample DNA is hybridized with 4 kinds of 8-baselength probes given below.

CCGTCGTT (SEQ ID NO: 2)

CCCGTCGT (SEQ ID NO: 3)

GGCCGTCG (SEQ ID NO: 4)

TGGCCGTC (SEQ ID NO: 5)

[0118] The probe shown in SEQ ID NO: 2 (hereinafter referred to as probe 2) is a complementary sequence to the 6th through 13th bases of the sample DNA. Similarly, SEQ ID NO: 3 (probe 3), SEQ ID NO: 4 (probe 4), and SEQ ID NO: 5 (probe 5) are complementary sequences to 5th through 12th, 4th through 11th, and 3rd through 10th bases of the sample DNA, respectively.

[0119] The temperature Tm, at which these probes are hybridized with the sample DNA, was measured using the DNA chip according to the present invention.

SEQUENCE LISTING

<110> HITACHI, LTD.

<120> Advanced Thermal Gradient DNA Chip (ATGC), the Substrate for ATGC, Method for Manufacturing for ATGC, Method and Apparatus for Biochemical Reaction, and Storage Medium

<130>

<140>

<141>

<150> JP-356433/1999

<151> 1999-12-15

<160> 7

<210> 1

<211> 17

<212> DNA

<213> Artificial Sequence

<223> sample DNA fragment

<400> 1

tgaccggcag caaaatg 17

<210> 2

<211> 8

<212> DNA

<400> 5

tggccgtc 8

<210> 6

<211> 17

<212> DNA

<213> Artificial Sequence

<223> sample DNA fragment

<400> 6

tgaccggtag caaaatg 17

<210> 7

<211> 17

<212> DNA

<213> Artificial Sequence

<223> sample DNA fragment

<400> 7

tgaccggaag caaaatg 17

Claims

1. A biochemical reaction detection chip, comprising a membranous substrate having an insulating property, a plurality of islands provided on one side of said substrate, and probe cells for immobilizing probes for detecting the biochemical reaction, said probe cell being provided on the other side of said substrate and at sites corresponding to the islands, wherein each island comprise a heater for heating individual island, a temperature detector for detecting the temperatures of individual island, a heater terminal for controlling the heating by the heater, a temperature detection terminal for outputting the result of detection by said temperature detector, wherein the heating by the heater is externally controlled through the heater terminal according to the output of the temperature detection terminal.

2. A biochemical reaction detection chip according to claim 1, wherein the membrane on said substrate is formed from a kind of material or a composite material selected from a group consisting of silicon nitride, silicon oxide, aluminium oxide and Ta_2O_5.

stopping heating process (a) or using a cooler.

18. A method according to claim 15, 16 or 17, wherein the biochemical reaction is the hybridization between polynucleotide and oligonucleotide, and the optimal temperature for the biochemical reaction is the melting temperature of double strand formed with the oligonucleotide and its complementary strand.

19. A method according to claim 18, wherein the polynucleotide is DNA in a sample, and the oligonucleotide is oligonucleotide probe of the biochemical reaction detection chip.

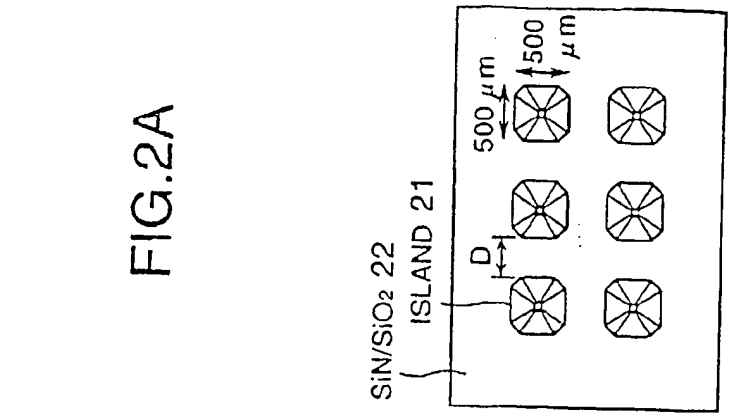
20. A computer-readable storage medium storing a program for executing a method for performing a plurarity of biochemical reactions in a plurarity of reaction systems simultaneously while controlling the temperature for each reaction system, the method comprising the steps of:

(a) heating all the reaction systems to a temperature higher than the optimal temperature for the biochemical reaction in each reaction system, and
(b) lowering the temperature of each reaction system to an optimal temperature for each biochemical reaction in each reaction system and maintaining the temperature for a certain period of time.

21. A computer readable storage medium having recorded thereon code components that, when loaded on a computer and executed will cause that computer to operate according to any one of claims 15 to 19.

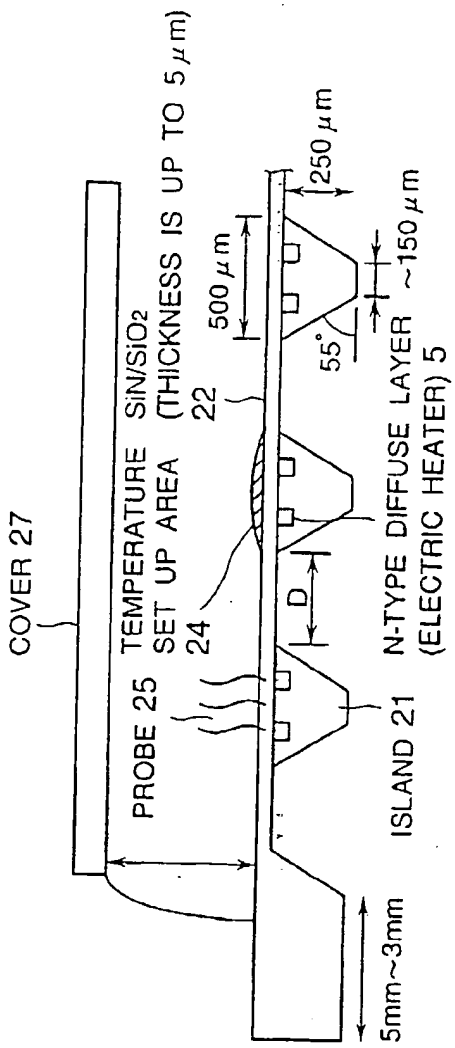
22. A computer program comprising program code means adapted to perform the method of any one of claims 15 to 19.

FIG.2A



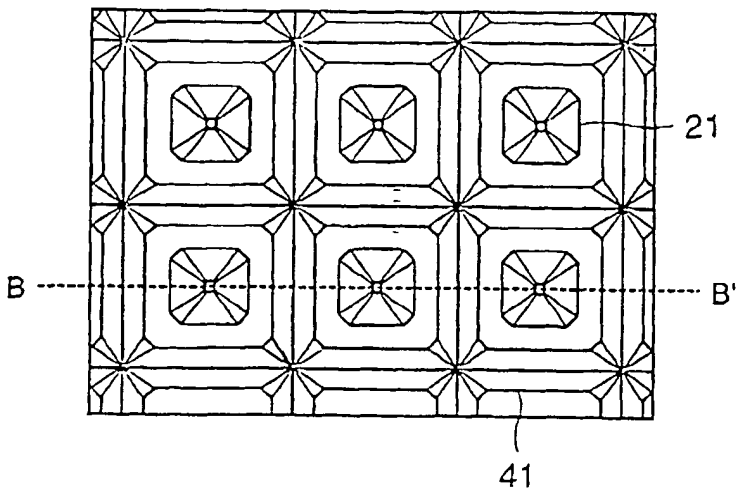
AN ENLARGED BACKSIDE PICTURE

FIG.2B



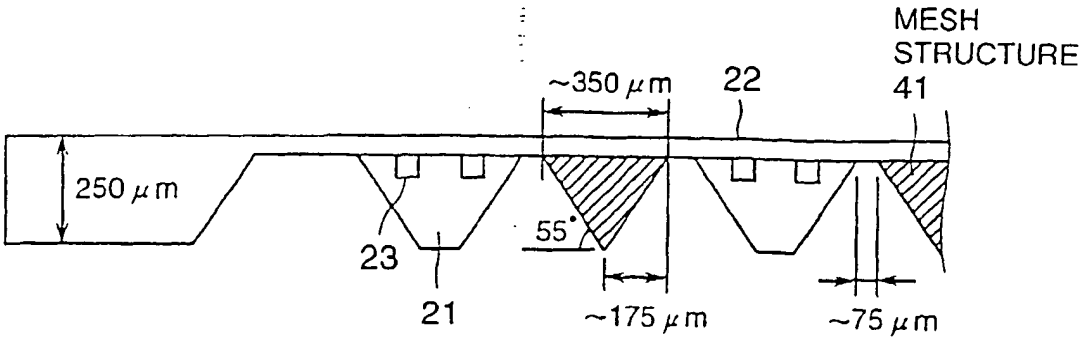
A CROSS SECTION VIEW AT A-A'

FIG.4A



AN ENLARGED BACKSIDE PICTURE

FIG.4B



A CROSS SECTION VIEW AT B-B'

FIG.6

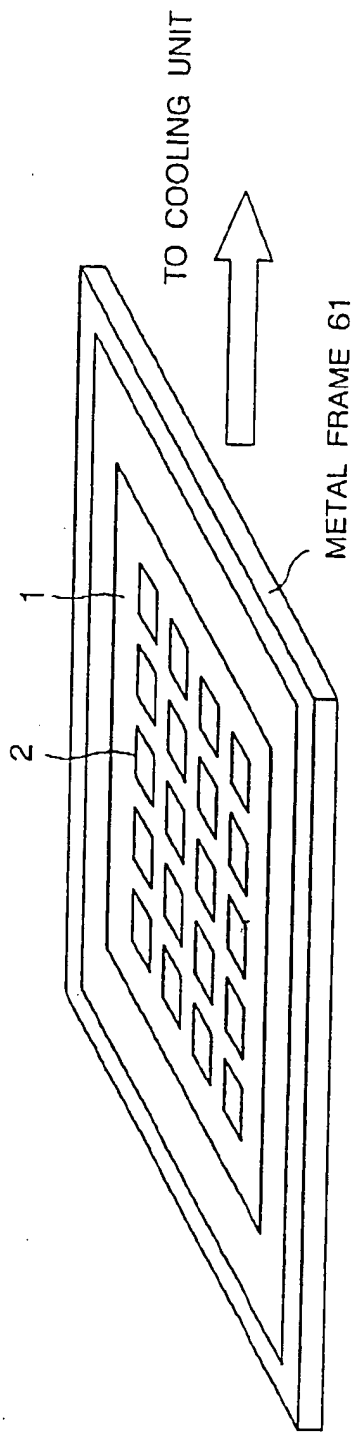


FIG.8

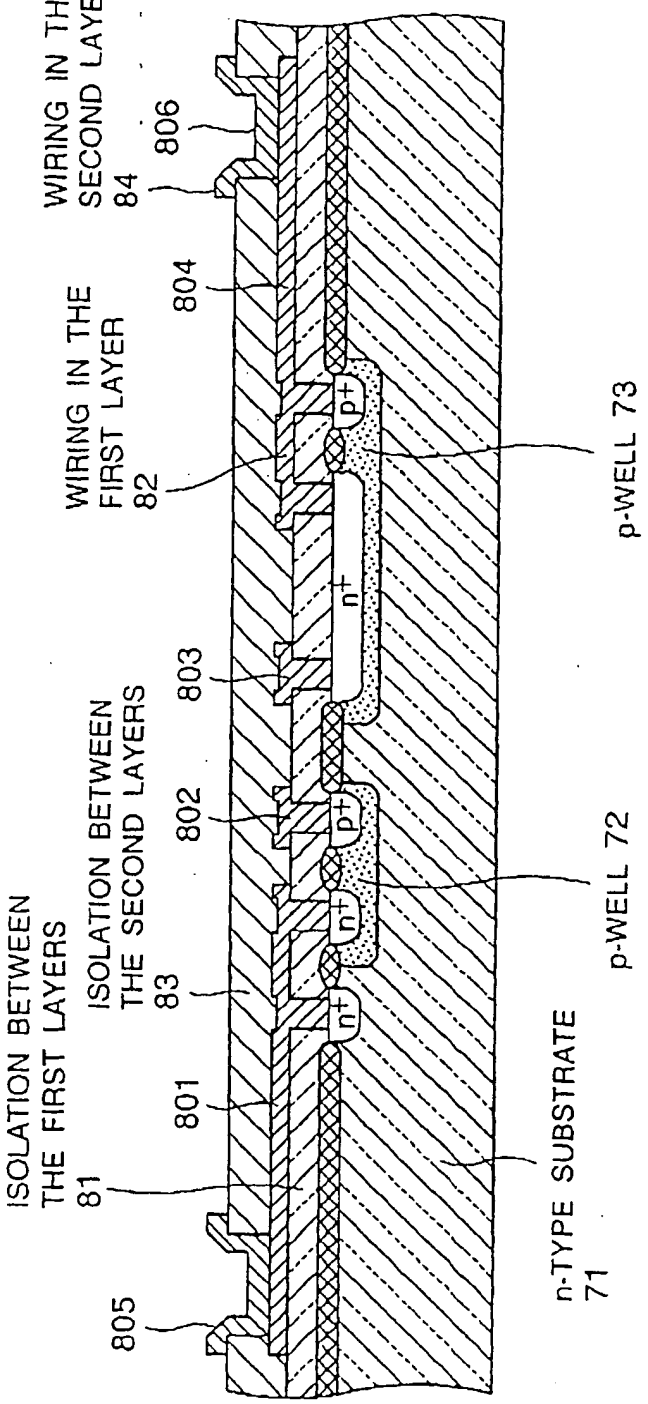


FIG.10

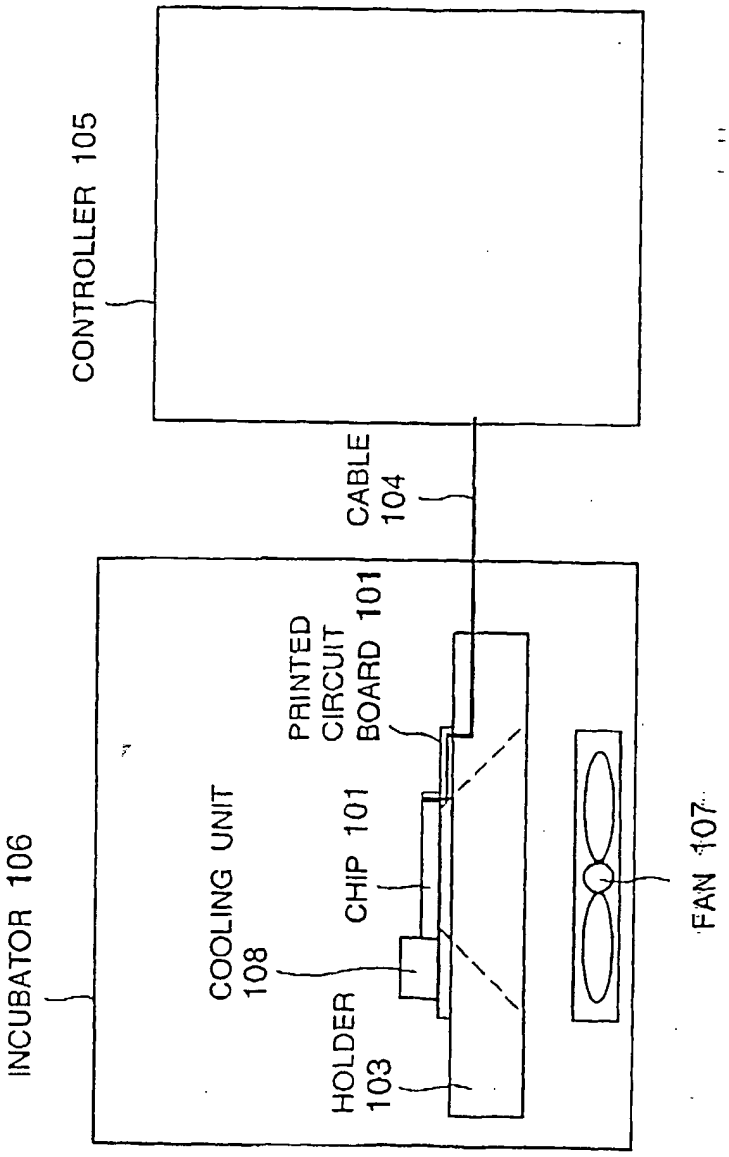


FIG.12A

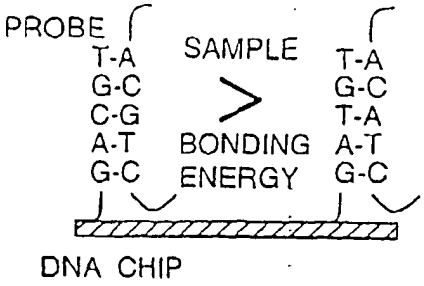


FIG.12B

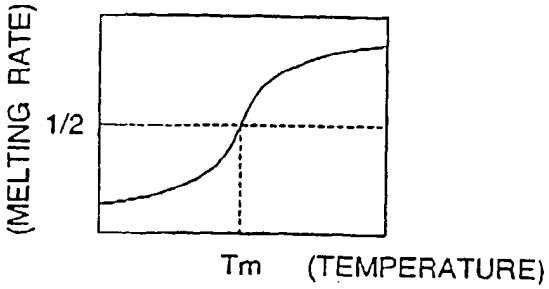


FIG.12C

8-MER PROBE EXAMPLE

	MIN.	MAX.	ΔTm
PROBE	ATATATAT	GCGCGCGC	
Tm *1	15.2	56.2	41.0

*1 : APPROXIMATE VALUE(%GC METHOD)

FIG.13

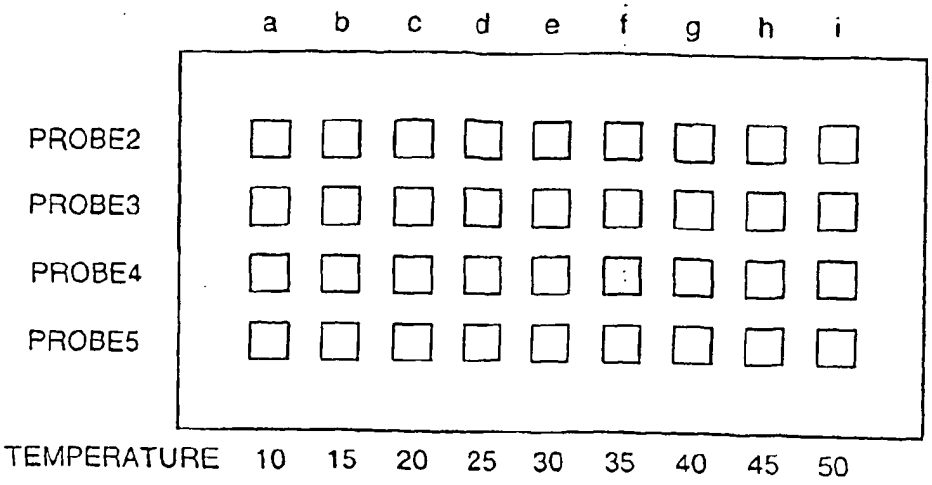


FIG.16

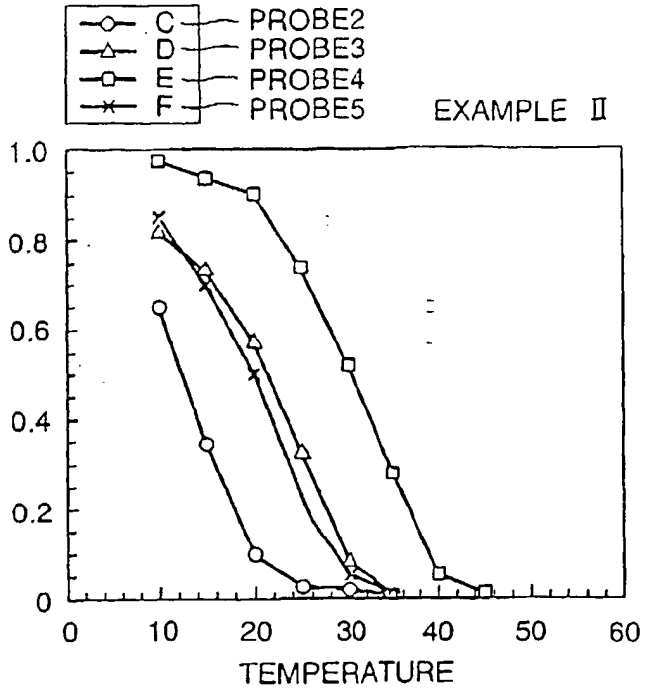


FIG.17

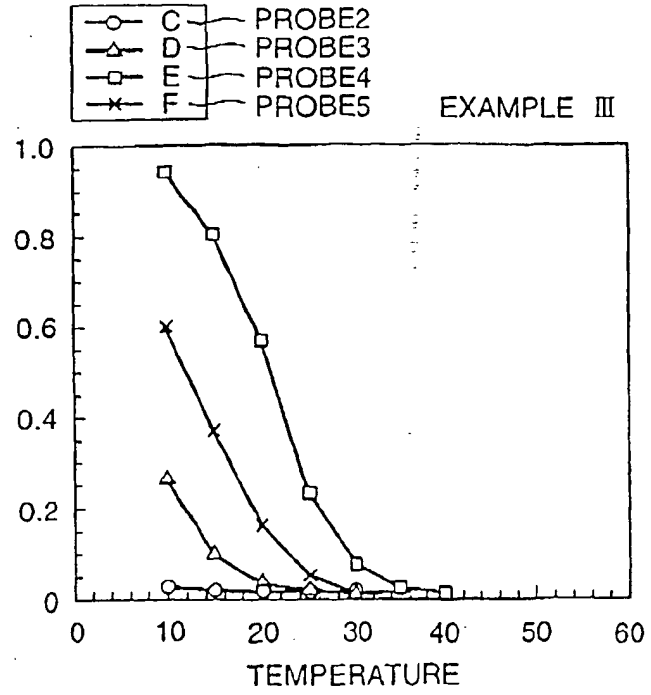
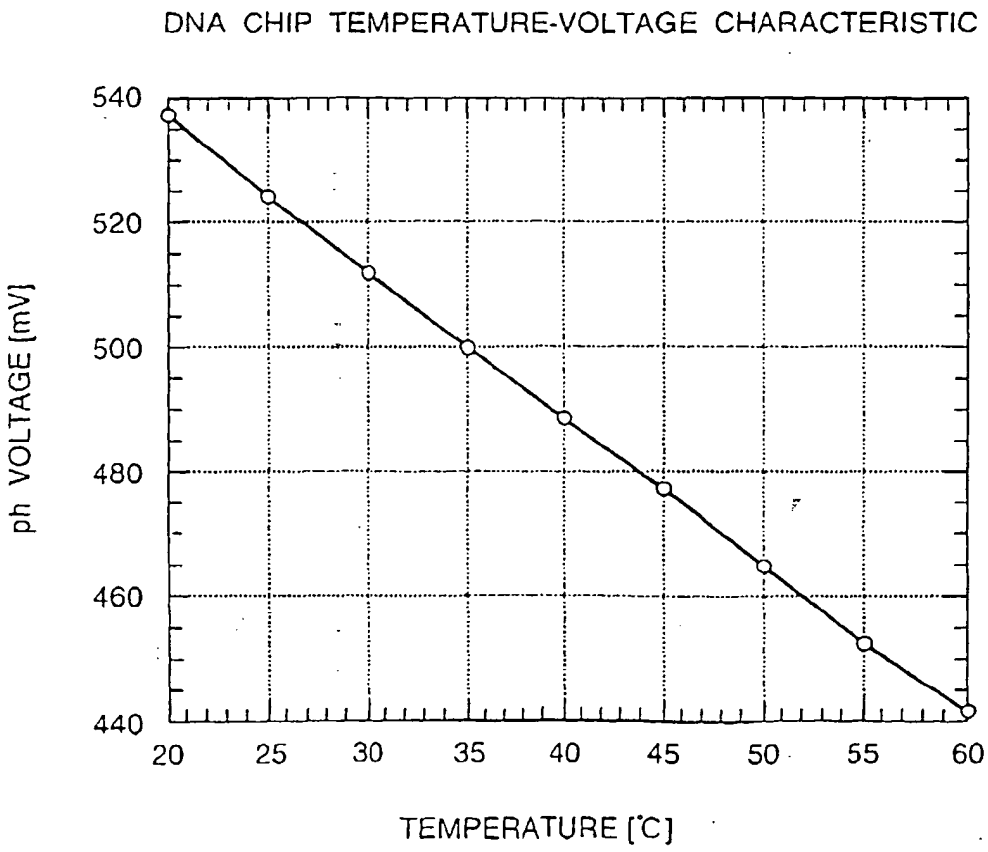


FIG.19



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 108 472 A3

(12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
29.10.2003 Bulletin 2003/44

(43) Date of publication A2:
20.06.2001 Bulletin 2001/25

(21) Application number: **00302224.1**

(22) Date of filing: **17.03.2000**

(51) Int Cl.[7]: **B01L 7/00**, B01L 3/00, B01J 19/00, C12Q 1/68

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **15.12.1999 JP 35643399**

(71) Applicant: **Hitachi, Ltd.**
Chiyoda-ku, Tokyo 101-8010 (JP)

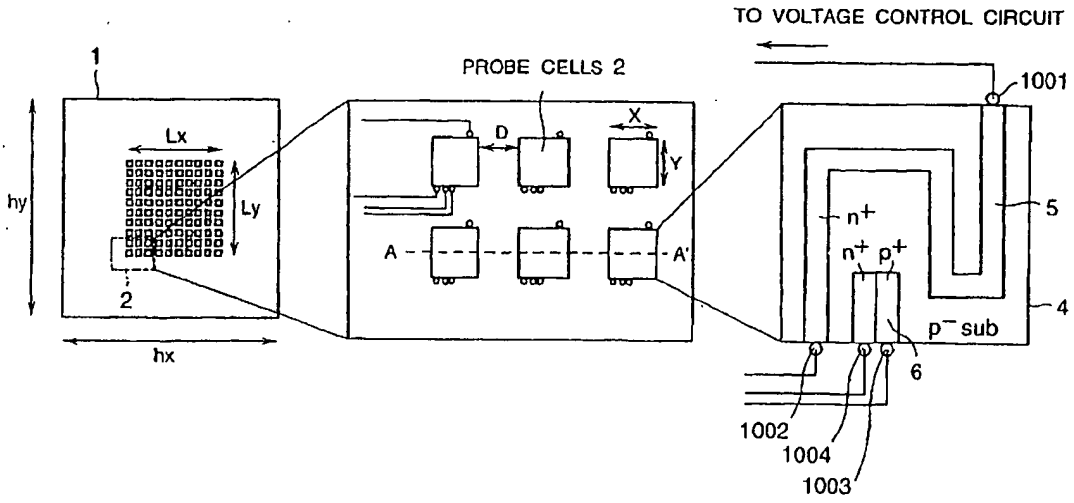
(72) Inventors:
- **Kajiyama, Tomoharu**
  Kokubunji-shi, Tokyo 185-0005 (JP)
- **Miyahara, Yuji**
  Kodaira-shi, Tokyo 187-0022 (JP)
- **Murakawa, Katsuji**
  Kodaira-shi, Tokyo 187-0045 (JP)

(74) Representative: **Nicholls, Michael John**
J.A. KEMP & CO.
14, South Square
Gray's Inn
London WC1R 5JJ (GB)

(54) Advanced thermal gradient DNA chip (ATGC), it's manufacture method and method for carrying out biochemical reactions

(57) A biochemical reaction detection chip capable of controlling the temperature for biochemical reactions including hybridizations and its substrate. The function of the chip is performed by comprising a plurality of islands of a heat conducting material on the membrane of the substrate, the islands being spaced from each other and individually provided with temperature controllers, and the probes immobilized on the substrate.

FIG.1A FIG.1B FIG.1C



EP 1 108 472 A3

European Patent Office

Application Number
EP 00 30 2224

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 30 2224

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

01-09-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5674742 A	07-10-1997	US 5639423 A	17-06-1997
		AU 5092193 A	29-03-1994
		EP 0711200 A1	15-05-1996
		JP 3002541 B2	24-01-2000
		JP 7508928 T	05-10-1995
		WO 9405414 A1	17-03-1994
		US 5646039 A	08-07-1997
WO 9850154 A	12-11-1998	US 6054277 A	25-04-2000
		WO 9850154 A1	12-11-1998
		US 6303288 B1	16-10-2001
US 6093370 A	25-07-2000	JP 2000279169 A	10-10-2000
		US 2002048771 A1	25-04-2002
		US 2002055119 A1	09-05-2002
		US 2002090641 A1	11-07-2002
		US 6218126 B1	17-04-2001
		US 6361953 B1	26-03-2002
		US 2001029017 A1	11-10-2001
US 5415839 A	16-05-1995	NONE	
WO 9846793 A	22-10-1998	AU 7103098 A	11-11-1998
		WO 9846793 A1	22-10-1998
		US 6548247 B1	15-04-2003

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82